Memorandum

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To: Michael McGuffin,
American Herbal Products Association

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Subject: Comments on NTP Technical Report on the Toxicology and Carcinogenesis of Goldenseal Root Powder (*Hydrastis canadensis*) in F344/N Rats and B6C3F1 mice

In this memo, we provide comments on the NTP Technical Report on the Toxicology and Carcinogenesis of Goldenseal Root Powder (*Hydrastis canadensis*) in F344/N Rats and B6C3F1 mice (NTP TR 562), which is scheduled for peer review on 25 February 2009. NTP's report concludes that there was clear evidence of carcinogenic activity of goldenseal root powder in male and female F344/N rats based on non-neoplastic effects in the liver, hepatocellular adenomas in both males and females, and the single hepatocellular carcinoma in a male rat. The report also concludes that there was some evidence of carcinogenic activity in male mice based on non-neoplastic effects in the liver, and hepatoblastomas and multiple hepatocellular adenomas in the liver.

These classifications, however, are not appropriate, because the one significant increase in carcinomas observed in the entire study (one hepatocellular carcinoma at the high dose in a male rat), is within historic control incidence. In addition, while increased incidences of hepatocellular adenomas were observed in both sexes of rats and in male mice, the adenomas were not associated with carcinomas. Elevation of adenomas without an associated increase in carcinomas, as compared to mere tumorigenicity, is much weaker evidence of carcinogenicity. For goldenseal, there are no excess hepatocellular carcinomas in mice, despite excess adenomas, casting doubt on the general presumption that the liver adenomas observed can progress to carcinomas. Thus the classifications of carcinogenic activity (based on liver neoplasms), are made without any compelling elevation in liver carcinomas. A detailed discussion is provided below.

Results of NTP Bioassay

Rats

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This 2-year feeding study found effects of goldenseal in the liver in two rodent models – F344/N rats and B6C3F1 mice. In F344/N rats, an increased incidence of hepatocellular adenomas was found at the highest dose in both males and females, as well as a single case of hepatocellular carcinoma at the highest dose of goldenseal (25,000 ppm) in the male rats (see Table 1). The incidence of hepatocellular adenomas was essentially flat until the high-dose group, although the dose-response trend was significant. Non-neoplastic effects of goldenseal were also observed, such as increased incidences of hepatocyte hypertrophy, hepatocyte degeneration, and eosinophilic foci, in both male and female rats. All three of these endpoints increased in a dose-dependent manner in male and female rats.

Table 1. Summary of Neoplastic Results from the NTP Goldenseal Bioassay

Dose (ppm)	0	3,000	9,000	25,000	Overall Trend
Male Rat Neoplastic Effects – NTP Clear Evidence					
Hepatocellular adenoma	1/50	1/50	2/50	10/50*	P<0.001*
Hepatocellular carcinoma	0/50	0/50	0/50	1/50	----
Combined adenomas/carcinoma	1/50	1/50	2/50	11/50*	P<0.001*
Female Rat Neoplastic Effects – NTP Clear Evidence					
Hepatocellular adenoma	0/50	0/50	1/50	8/50*	P<0.001*
Male Mouse Neoplastic Effects – NTP Some Evidence					
Hepatoblastoma	1/50	2/50	1/50	6/50	P=0.016
Multiple hepatocellular adenoma	3/50	5/50	11/50	18/50	not provided
Hepatocellular adenoma	22/50	16/50	23/50	29/50	P=0.03
Female Mouse Neoplastic Effects– NTP No Evidence					

** Significantly increased compared to control or significant increase in overall trend.*

Note: Data from page 12 and Table C2 in the NTP report.

Mice

The NTP study reported no significant increases in neoplastic or non-neoplastic effects in female mice, but an increased incidence of hepatoblastomas and multiple hepatocellular adenomas in male mice (see table on page 12 of NTP report). However there is no statistical analysis of either the multiple hepatocellular adenomas or single hepatoblastomas in Table C2 in Appendix C, which presents the statistical analysis of primary neoplasms in male mice. Rather, the category of Liver: Hepatocellular Adenomas in Table C2 presents the results for the combined incidence of animals with single hepatocellular adenomas and animals with multiple hepatocellular adenomas (see Table C1)[1]. However

[1] In Table C1, the line for hepatocellular adenoma is for animals with single tumors, and the line below is for animals with multiple adenomas (meaning more than one adenoma per liver). These numbers are added together to yield the total animals

even combining the two categories does not result in a significant increase over controls at any individual dose. While the trend is significant, this is very weak evidence of "carcinogenic activity" (the goal of the testing), especially since there is neither a significant increase in hepatocellular carcinoma incidence, nor a dose-response (see Table C1). In addition, animals with a single hepatocellular adenoma had no increased incidence when compared to controls (the incidence for all three doses groups was lower than in the controls).

We do not know how many hepatocellular adenomas the mice with multiple adenomas had (the data are not provided in report), so we cannot assess the total count *vs* dose. Nonetheless, the data suggest that there are not more mice with adenomas at higher doses, just a tendency for more adenomas in mice that have them. It is not clear why this clustering within animals happens, but it might be evidence of the need for prior liver toxicity as a causative element. The reliance in the NTP report on increased multiplicity, not on increased incidence, as evidence of tumorigenicity is unusual.

Adenomas are benign neoplasms, so their increased incidence is not direct evidence of carcinogenicity *per se*. The use of adenomas to evaluate carcinogenic activity rests on the concern that the adenomas could become malignant. The generation of liver adenomas, which are known to be able to progress to malignancy (*i.e.*, to carcinomas), is regarded as evidence of potential carcinogenicity. NTP's analysis, in which they combined hepatocellular adenomas and carcinomas for statistical analysis, reflects this approach. Elevation of adenomas without an associated increase in carcinomas, as compared to mere tumorigenicity, is much weaker evidence of carcinogenicity. For goldenseal, there are no excess hepatocellular carcinomas in mice, despite excess adenomas, casting doubt on the general presumption that the liver adenomas observed can progress to carcinomas.

Male mice also had an increased rate of hepatoblastomas, but although the trend was significant, the dose-response was flat until the high-dose group, and no single dose was associated with a significant increase as compared to controls. As with hepatocellular adenomas, the incidence rates shown in Table C2 under the category of Liver: Hepatoblastomas are the combined incidence of animals with single hepatoblastomas and animals with multiple hepatoblastomas (see Table C1). As shown in Table C2, male mice had no significant positive trends or significantly increased incidences of either hepatocellular carcinoma, hepatocellular adenoma or carcinoma combined; hepatocellular carcinoma or hepatoblastoma combined; or hepatocellular adenoma, carcinoma or hepatoblastoma combined.

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with adenomas. For example, for the high dose, there were 11 mice with a single adenoma and 18 with multiple adenomas, for a total of 29 animals with (at least one) adenoma; this total is presented in Table C2 as Liver hepatocellular adenoma.

Based on these findings, NTP concluded that there was clear evidence of carcinogenic activity of goldenseal root powder in male and female F344/N rats and that there was some evidence of carcinogenic activity in male, but not female, B6C3F1 mice. In addition to the patterns and statistical significance of the dose-responses relied on in the NTP report, a number of factors bear consideration in evaluating the carcinogenicity of goldenseal. Since a single liver malignancy occurred only at the high dose in a male rat, and the evidence suggests that the adenomas do not progress to malignancy over the lifetime of the rodents (either in rats or mice), it is important to consider the mode of action, non-neoplastic lesions, time-to-first tumor, the evidence regarding mutagenicity, historical background rates of tumors, and the protective role of goldenseal for certain health endpoints. These topics are discussed in the remainder of this memo.

Mode of Action

Cytotoxicity is a generally accepted MOA for a number of nongenotoxic rodent carcinogens (Anderson *et al*., 1998; Sonich-Mullin *et al*., 2001, both as cited in Holsapple *et al*., 2006). In this MOA, a hepatocyte cytotoxicant produces continual hepatocyte death, leading to persistent regenerative growth, allowing more opportunities for DNA mutations, leading ultimately to tumors. However to make a determination that such a mechanism is occurring, other MOAs (*e.g.*, DNA reactivity) must be ruled out as contributing significantly to hepatocarcinogenesis. In addition, it is important to establish parallel dose response for the key events (*i.e.*, cytotoxicity and proliferation) and tumors, as well as a specificity of the key events and the tumor response (Holsapple *et al*., 2006). This type of detailed information was not available for the 2-year study in the NTP report. We note that this mechanism is associated with a non-linear dose-response that likely has a threshold. Such a mechanism is often dependent on similar metabolism in the animal model and humans (*e.g.*, metabolic activation of CYP2E1 in the case of chloroform) (Holsapple *et al*., 2006).

The metabolism of goldenseal or its components is not understood, although for one of the components of goldenseal, berberine, there are qualitative differences in human and rat metabolism (as shown in Figure 2 on page 24 of the NTP report). Berberine is one of the alkaloids in goldenseal; there is apparently no information regarding the metabolism of goldenseal as a whole. As noted by NTP, goldenseal and/or its constituents affect a number of cytochrome P450 enzymes (*e.g.*, CYP2E1, CYP1A2, CYP2D6,, CYP3A4/5), so differences in rat and human metabolism could be very important in terms of affecting toxicity.

Non-neoplastic Effects *vs.* Neoplastic Effects

There is uncertainty about whether the non-neoplastic events observed in rats progress to neoplastic events. Thus, more information is needed to assess the progression of non-neoplastic to neoplastic effects.

Owing to a lack of data, we cannot evaluate whether non-neoplastic events observed in rats progress to neoplastic events. The NTP Report does not provide individual animal pathology tables with which to associate non-neoplastic and neoplastic responses on an animal-by-animal basis, and in any case, detailed histopathological observation of association of neoplastic tissue with non-neoplastic tissue pathology in individual rat livers would be needed to clarify this. It is nonetheless clear that numerous non-neoplastic effects were found in the livers of rats (see Table 2) at substantial incidence rates. For instance, hepatocyte hypertrophy and hepatocyte degeneration were found in both male and female rats in a dose-dependent manner, although in two cases the maximum incidence was at the mid-dose (hepatocyte hypertrophy and hepatocyte degeneration in male rats). These non-neoplastic incidence rates were much higher than the rates for the neoplastic effects observed in the same animals, and in no instance did tumors appear at a dose without a substantial incidence of non-neoplastic toxicity in the liver. Although neoplastic effects were observed in rats and male mice, there was no clear relationship between the neoplastic and the non-neoplastic events observed.

Table 2. Summary of Non-Neoplastic Results from the NTP Bioassay

Dose (ppm)	0	3,000	9,000	25,000
Male Rat Non-Neoplastic Effects				
Hepatocyte hypertrophy	0/50	19/50*	31/50*	27/50*
Hepatocyte degeneration	0/50	22/50*	30/50*	19/50*
Liver: Eosinophilic focus	4/50	5/50	25/50*	28/50*
Female Rat Non-Neoplastic Effects				
Hepatocyte hypertrophy	2/50	10/50*	27/50*	38/50*
Hepatocyte degeneration	1/50	2/50	12/50*	24/50
Liver: Eosinophilic focus	2/50	24/50*	29/50*	22/50*
Male Mouse Non-Neoplastic Effects				
Liver: Eosinophilic focus	7/50	14/50	14/50	24/50*

* *Significantly increase compared to unexposed controls ($P \leq 0.05$)*

Time-to-First-Tumor

Time-to-first-tumor should decrease as dose increases, if the agent tested is responsible for the excess tumors. However for the excess hepatocellular adenomas, hepatocellular adenomas or carcinomas,

and hepatoblastomas identified in male mice, there was a lack of concordance between the time-to-first-tumor and dose. The time-to-tumor for hepatocellular adenomas was smallest in the low-dose group (not the high-dose group, as would be expected). For hepatocellular adenomas or carcinomas, the shortest time-to-first-tumor occurs in the mid-dose group (551 days), which is almost identical to the low-dose group, 555 days (see Table 3). The time-to-first-tumor for the high-dose group was 595 days. For hepatoblastomas in male mice, the shortest time-to-first-tumor was in the low-dose group (673 days), while the longest time to first tumor was in the controls and the mid-dose group (729 days); it was 722 days in the high-dose group.

No Evidence of Mutagenicity

There was no evidence to support mutagenic effects of goldenseal. NTP provided evidence that it was not mutagenic in *Salmonella typhimurium* or *Escherichia coli* tester strains, with or without liver S9 metabolic activation enzymes. Frequencies of micronucleated erythrocytes were not increased in the peripheral blood samples from mice exposed to goldenseal root powder. Berberine chloride, a component of goldenseal powder, was non-mutagenic in *Salmonella typhimurium,* with or without rat or hamster liver S9 metabolic activation enzymes. These negative findings suggest that goldenseal is non-mutagenic. In the absence of mutagenicity, toxicity effects, including cancer, are believed to occur only above a threshold dose. The dose-response data for liver adenomas, which demonstrate a hockey stick-shaped dose-response curve, are consistent with a threshold response.

Table 3. Time-to-First Tumor for Hepatocellular Adenomas/Carcinomas in Male Mice (days)

Dose (ppm)	**0**	**3000**	**9000**	**25,000**
Hepatocellular adenoma	677	555	666	596
Hepatocellular adenoma or carcinoma	681	555	551	596
Hepatoblastoma	729	673	729	722

Note: Data from Table C2 in the NTP report.

The hepatoblastomas, in particular, occurred late in life and were not life-shortening.

Consideration of Historical Background Rates of Liver Tumors

Historical data on the incidences of spontaneous neoplasms in control animals are used in the assessment of carcinogenicity trials to avoid false positive results (Tennekes *et al*., 2004). While the NTP report incorporated historical incidence of liver tumors from four feeding studies, many more have been conducted. A more comprehensive presentation of background rates would be helpful in interpreting the goldenseal bioassay data. We compared results in the bioassay to both the historical data provided by NTP in their report and to data provided in the scientific literature.

For male F344/N rats, the background rate of hepatocellular carcinoma reported by NTP report in the goldenseal study was 0/50, as compared to the historical background rate of hepatocellular carcinomas in male F344/N rats reported in the literature, which range from 0-2%, averaging 0-0.3% (Tennekes *et al*., 2004; Chandra and Frith, 1992). Thus the single hepatocellular carcinoma found in male rats in the NTP goldenseal study , which was 2% (1 out of 50 animals), was within the range of historical background rates.

Protective Role of Goldenseal

Increased incidences of tumors are of concern, but if goldenseal also induces decreases in other tumor responses, it may also be protective. When evaluating effects of a chemical or any agent, possible toxicological as well as the potential protective role of the agent should be taken into consideration. Although the NTP study found increased incidences for several endpoints examined in the liver, it also found decreased incidences of many other endpoints. For example, incidence rates of pancreatic islets adenoma, pancreatic islets adenoma or carcinoma, thyroid gland adenoma, and thyroid gland adenoma or carcinoma decreased in male rats exposed to goldenseal at all doses. Additionally, incidence rates of a number of neoplasms were decreased at all doses in female rats exposed to goldenseal (*e.g.*, mononuclear cell leukemia, clitoral gland carcinoma, all categories of mammary gland tumors, thyroid gland adenoma, and benign and/or malignant neoplasms in all organs). Some endpoints had a significant decrease at all doses and a significant negative trend, as well (*e.g.*, mammary gland fibroadenoma; mammary gland fibroadenoma, adenoma, or carcinoma). Male mice had decreased incidences of Harderian gland adenoma and carcinoma, and alveolar/bronchiolar carcinoma. Although there were some increased incidences of neoplastic events in the rat liver, there were many cases of decreased neoplastic effects, including carcinogenic effects, in multiple organs. These findings suggest that goldenseal has protective effects with respect to neoplastic effects including cancer.

Moreover, non-neoplastic effects were also decreased in various organs, with exposure to goldenseal. For example, decreased cardiomyopathy was detected in both male and female rats. Decreased incidence of cellular infiltration of histiocytes in the lung in male rats, as well as decreased cyst in pars distalis of the pituitary gland in female rats were found. These results add further support for goldenseal having a protective role for some endpoints.

Conclusion

NTP's report concludes that there was clear evidence of carcinogenic activity of goldenseal root powder in male and female F344/N rats based on non-neoplastic effects in the liver, hepatocellular adenomas in both males and females, and the single hepatocellular carcinoma in a male rat. The report also concludes that there was some evidence of carcinogenic activity in male mice based on non-neoplastic effects in the liver, and hepatoblastomas and multiple hepatocellular adenomas in the liver. However, these classifications are not appropriate for the following reasons:

- Only one significant increase in carcinomas was observed in the entire study (one hepatocellular carcinoma at the high dose in a male rat), and this incidence is within historic control incidence.
- Both the increased incidence of hepatocellular adenomas in male and female rats, and the increase in combined incidence of hepatocellular adenomas or carcinomas in male rats, are only significant at the highest dose tested. The associated dose-response curves resemble hockey sticks, which is consistent with a threshold response. A threshold response is also supported by the lack of mutagenicity of goldenseal, as reported by NTP.
- While hepatocellular adenomas were observed in both sexes of rats and in male mice, these adenomas were not associated with carcinomas.
- In male mice, the increased incidence of single adenomas or the combined incidence of animals with single and multiple adenomas, was not significant at any single dose (the statistical analysis is not provided for multiple hepatocellular adenomas).
- Similarly the increased incidence of hepatoblastomas in male mice was not significant at any single dose.
- The increased incidence of hepatocellular tumors in mice consists solely of benign tumors.
- There was a lack of concordance between the time-to-first-tumor and dose for hepatocellular adenomas, hepatocellular adenomas or carcinomas, and hepatoblastomas in male mice. In no case (related to neoplastic effects in the liver) was the shortest time-to-first-tumor associated with the high dose.
- There is no clear relationship between the neoplastic and the non-neoplastic events observed in rats and male mice.

- Goldenseal may have an overall protective effect with respect to neoplastic effects, as indicated by decreased incidence rates of multiple endpoints in both male and female rats, and in male mice.

References

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